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SENESCENCE OF RICE LEAVES

X. The Effects of Metal Chelators

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Abstract

The effects of some metal chelators on the senescence of rice leaf segments were studied in terms of chlorophyll loss and amino nitrogen accumulation. Metal chelators showed a differential action on senescence in the dark and light. Metal chelators effectively retarded the senescence in the dark. Among four chelators tested, α, α'-dipyridyl was the most effective chelator in retarding dark senescence. Ethylenediaminetetraacetic acid retarded dark senescence only at concentrations above 10 mM. All metal chelators tested promoted senescence in the light. Since the promotion of chlorophyll loss by metal chelators in the light was rapid and more effective than that of proteolysis, it seems two processes are involved in chlorophyll loss; one is normal senescence, the other is irreversible photooxidation. The possible mechanisms related to the effects of metal chelators are discussed.

Introduction

Metal chelators, such as α, α'-dipyridyl (DP), 8-hydroxyquinoline (HQN), salicylaldoxime (SAL) and ethylenediaminetetraacetic acid (EDTA), have been shown to retard the dark senescence of detached leaves (Chua, 1970; Kotaka and Krueger, 1969; Pjon, 1982; Rameshwar and Steponkus, 1970; Tetley and Thimann, 1975). However, when leaf segments were treated with metal chelators under light condition, acceleration of senescence was observed (Holden, 1972; Pjon, 1982; Thimann, 1982). Recently, we reported that straight-chain alcohols showed a differential action on senescence of rice leaves in the dark and light (Cheng and Kao, 1984). Therefore, it would be interesting to know whether metal chelators also show a differential effect on the senescence of rice leaves in the dark and light.

Materials and Methods

Rice (*Oryza sativa* cv. Taichung Native 1) seedlings were cultured as previously

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(Kao 1980b). The apical 3 cm of the third leaves of 8-day-old seedlings was used for experiments. Ten rice leaf segments were floated on 10 ml of test solution in a 50-ml flask. The flasks were then incubated at 27°C either under light (14Wm^{-2}) or in darkness. Chelator solutions were adjusted to pH 7 with NaOH or HCl before use. Chelators were of the highest purity that was readily available commercially.

Chlorophyll and α-amino nitrogen were extracted and determined as described before (Kao, 1980a, b), and expressed as A_{665} and A_{570} per ten segments, respectively.

Results

The senescence of excised rice leaves was followed by measuring the decrease of chlorophyll and the increase of amino nitrogen. Fig. 1 shows the time courses of chlorophyll and amino nitrogen levels of leaf segments floating on water or tested metal chelator solutions in darkness. It is clear that DP, HQN and SAL are all effective in retarding dark senescence and DP is the most effective metal chelator.

It has been previously shown that DP at concentration up to 3 mM did not promote senescence of detached oat leaves in the light (Tetley and Thimann, 1975). This experiment was repeated with rice leaf segments using DP, SAL and HQN. The results are presented in Fig. 2. Metal chelators strongly promoted senescence in the light. HQN was the most effective chelators in promoting senescences of rice leaf segment in the light. The promotion of chlorophyll loss by metal chelators was very rapid which was evident at one day after incubation. However, the promotion of proteolysis, as measured by amino nitrogen, was less effective and only

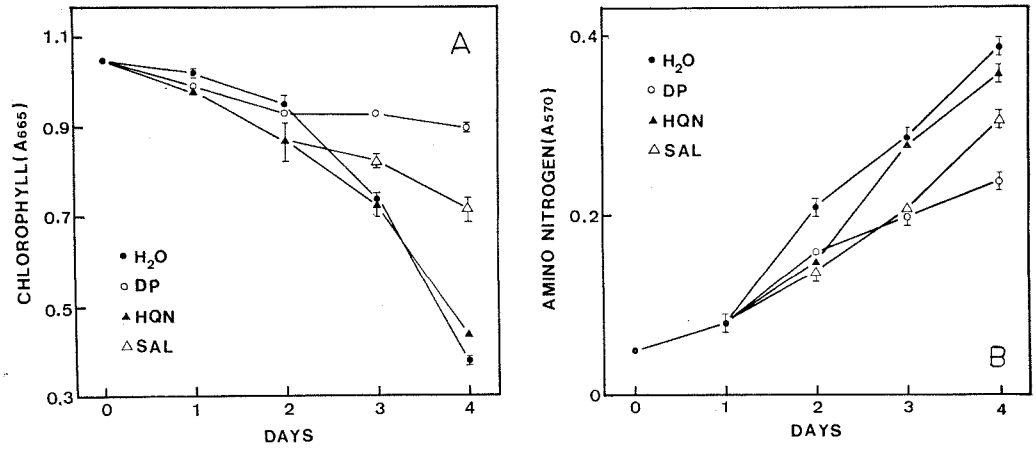

Fig. 1. Effect of DP (0.1 mM), HQN (1 mM) and SAL (1 mM) on chlorophyll (A) and amino nitrogen (B) contents of rice leaf segments under dark condition.

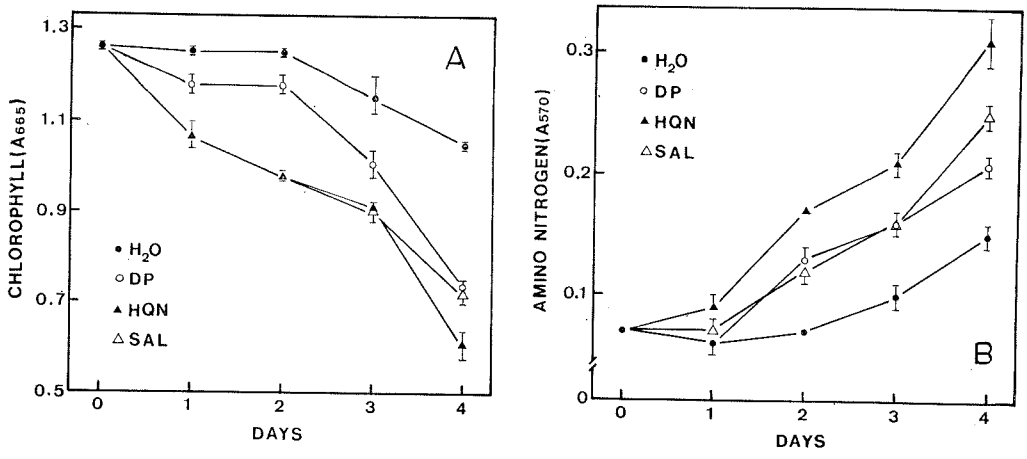

Fig. 2. Effect of DP (0.1 mM), HQN (1 mM) and SAL (1 mM) on chlorophyll (A) and amino nitrogen (B) contents of rice leaf segments under light condition.

occurred at 2 days after incubation. This indicates that, in addition to the normal mechanism, an irreversible photooxidation process may be involved in chlorophyll loss in rice leaves treated with metal chelators under light condition.

Generally, EDTA retarded dark senescence of rice leaf segments only at concentrations above 10 mM, which is much higher than the concentration required to promote senescence of rice segments in the light (Figs. 3 and 4). Since chlorophyll loss was more effectively promoted by EDTA than proteolysis, the photooxidation process may also be involved in chlorophyll loss in leaf segments treated with EDTA in the light.

Discussion

Most of the work concerning the effects of metal chelators in senescences of detached leaves was studied in terms of the loss of chlorophyll (Chua, 1970; Holden, 1972; Kotaka and Krueger, 1969; Pjon, 1982; Rameshwar and Steponkus, 1970; Tetley and Thimann, 1975). In this investigation, we used chlorophyll loss, and proteolysis, as measured by amino nitrogen, as indices to study the effect of metal chelators. Results shown in this paper, in general, confirm those reported earlier. Metal chelators showed a differential action on senescence of detached rice leaves in the dark and light.

Using oat leaves, Thimann *et al.* (1982) found that metal chelators promoted chlorophyll loss, but prevented proteolysis characteristic of senescence under light condition. However, our results showed that metal chelators promoted both chlorophyll loss and proteolysis of rice leaf segments is the light (Fig. 2). This discrepancy may be due to the different plant materials used.

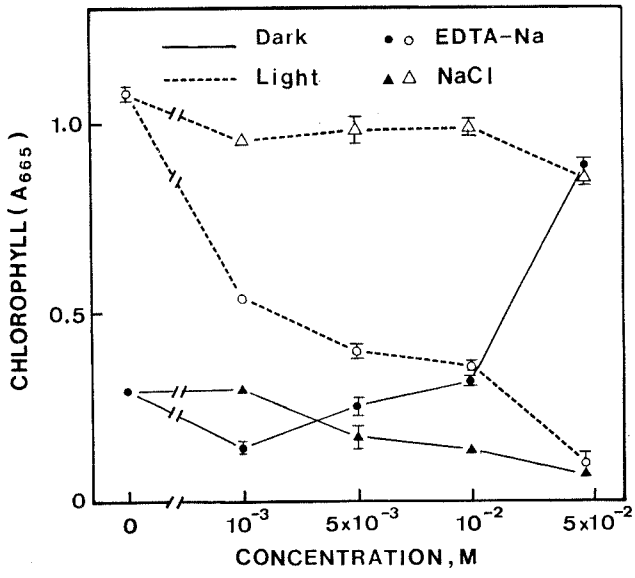

Fig. 3. Effects of EDTA concentrations on chlorophyll content of rice leaf segments. Chlorophyll was determined after 4 days.

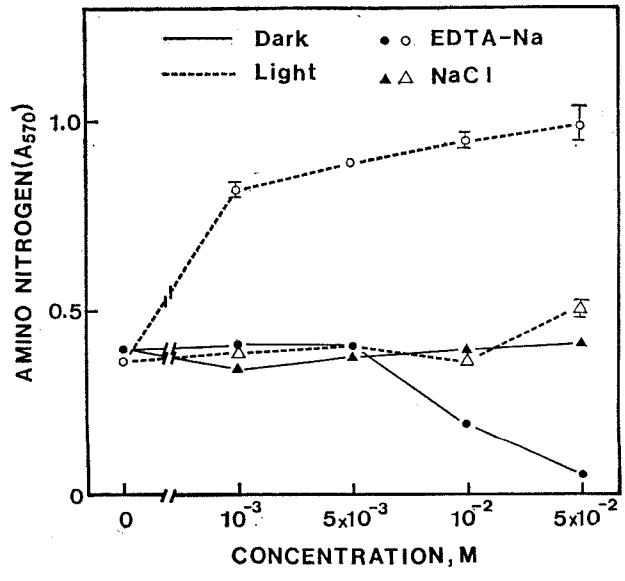

Fig. 4. Effects of EDTA concentrations on amino nitrogen content of rice leaf segments. Amino nitrogen was determined after 4 days.

The promotion of chlorophyll loss was either more rapid or more effective than that of proteolysis by metal chelators under light condition. Therefore, the chlorophyll loss of rice leaf segments treated with metal chelators in the light seems to be comprised of two processes; one is normal senescence and the other is photooxidation. This conclusion can be supported by the recent work of Pjon (1982), who reported that reducing agent, such as ascorbate, effectively inhibited the chelator-induced chlorophyll degradation of maize and hydrangea in the light.

One of the early events in leaf senescence is well-documented rise in protease activity (Thimann, 1980). The action of metal ions on dark senescence may be *via* chelation of metal ion in protease molecules which resulted in the inhibition of protease activity. It is not clear from our observation what metal ion is being chelated. The effect can not be due to the chelation of Ni^{2+} and Co^{2+}, since chlorides of these metals clearly retard dark senescence of rice leaf segments (Kao and Yu, 1981).

Cycloheximide, a protein synthesis inhibitor, was reported to retard dark senescence of rice leaf segments (Kao, 1978), suggesting the synthesis of new protein is necessary for the senescence to proceed. Since DP has been shown to prevent the hydroxylation of proline (Barnett, 1970; Hurych and Chvapil, 1965), it is possible that DP retards dark senescence *via* inhibiton of the synthesis of specific protein required for senescence.

Etiolated leaves generally fail to produce chlorophyll in the dark and synthesize chlorophyll only when treated with light. Adamson *et al.* (1980) recently reported that continuous synthesis of chlorophyll occurred in *Tradescantia albiflora* on transferred to darkness. In etiolated leaves, DP has been shown to synthesize protochlorophyllide and several prophyrin intermediates in the presense of chlorophyll biosynthesis precursor, δ-amino levulinic acid (Duggan and Gassman, 1974; Granick, 1961; Hendry and Stobart, 1978; Vlcek and Gassman, 1979). These facts seem to suggest that DP may not only retard chlorophyll degradation but also promote chlorophyll synthesis in green rice leaves in the dark.

The chlorophyll retarding effect of HQN was thought to resemble of cytokinins (Chua, 1970). However, it seems unlikely based on the evidence that HQN did not cause cell division in tissue culture bioassay (Rameshwar and steponkus, 1970). Furthermore, cytokinins retarded chlorophyll loss both under dark and light conditions (Kao and Yang, 1984), which is not the case of HQN (Figs. 1A and 2A).

The increased chlorophyll degradation in the light by metal chelators may result from its action in removing magnesium from the pyrrole rings of chlorophyll molecules, as suggested by Kotaka and Krueger (1969), Holden (1972) and Tetley and Thimann (1975). This may result in decomposition of chlorophyll either by direct photolysis, or by enzyme-linked photodecomposition.

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水稻葉片老化之研究

(十) 金屬抓合劑之效應

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本研究主要探討四種金屬抓合劑對水稻切離葉片老化之影響。金屬抓合劑對水稻葉片組織在暗中與光線下老化之效應不同。金屬抓合劑延緩切離葉片在黑暗下之老化。α,α'-dipyridyl 是四種抓合劑中最具延緩效果之抓合劑。Ethylenediaminetetraacetic acid 只有在濃度大於 10 mM 時才能延緩切離葉片在黑暗中老化。所有供試之抓合劑都促進切離葉片在光線下老化。由於抓合劑促進葉綠素之分解效應較促進蛋白質與分解效應爲大而且快速，因此於光線下抓合劑促進葉綠素分解之效應可能包括二種過程，一爲正常之老化，二爲不可逆之光氧化反應。抓合劑影響老化可能機制在本文內有詳細之討論。